

Molecular Markers Useful for Detecting Resistance to Brown Stem Rot in Soybean

K. L. E. Klos, M. M. Paz, L. Fredrick Marek, P. B. Cregan, and R. C. Shoemaker*

ABSTRACT

Brown stem rot (BSR) causes vascular and foliar damage in soybean [*Glycine max* (L.) Merr.]. Identification of plants resistant to BSR by inoculation with *Phialophora gregata* (Allington & W.W. Chamberlain) W. Gams is laborious and unreliable because of low heritability. Molecular markers linked to the resistance gene could be used to screen for resistant individuals and hasten the development of BSR resistant genotypes. The objective of this study was to develop molecular markers for efficient identification of BSR resistant plants in a breeding program. Seventeen resistant and 29 susceptible cultivars and plant introductions as well as recombinant inbred lines derived from a cross between BSR 101 and PI 437.654 were assayed by PCR-based markers derived from RFLPs K375I-1 and RGA2V-1, Satt244, or developed from bacterial artificial chromosome (BAC) sequences. The DNA markers that were developed tag the BSR locus and are informative in a diverse range of soybean germplasm. Markers detected different banding patterns between resistant and susceptible genotypes. The PCR-based markers will most likely be useful in screening for BSR resistance and allow soybean breeders to transfer rapidly resistance derived from *Rbs₃* to improved cultivars or soybean lines. The markers are relatively easy-to-use, inexpensive, and highly informative. Soybean breeding efforts can now be designed to incorporate the use of marker information when parental genotypes possess contrasting banding patterns.

BROWN STEM ROT is a devastating fungal disease of soybean (*Glycine max*) caused by *Phialophora gregata*, a soil-borne fungus. The pathogen infects host plants through the roots and causes vascular and foliar injury to the susceptible plants (Allington and Chamberlain, 1948; Mengistu and Grau, 1986). The disease is prevalent in soybean producing regions of the northern USA and Canada (Sinclair and Backman, 1989) and has been estimated to cause a yield reduction of over 20 million bushels each year in the north central states alone, depending upon environmental conditions (Doupnik, 1993).

Host resistance is the main means of controlling BSR. Plant introductions (PIs) have been identified as sources of non-allelic BSR resistance genes: PI 84946-2 for *Rbs₁* (Sebastian and Nickell, 1985) and *Rbs₃* alleles (Eathington et al., 1995); PI 437.833 for *Rbs₂* (Hanson et al., 1988); and PI 437.970 for *Rbs₃* (Willmot and Nickell,

1989). Other resistance genes may exist. Multiple genes may control BSR resistance in Asgrow A3733 which are not derived from known sources of resistance (Waller et al., 1991). Nelson et al. (1989) identified three resistant lines: PI 424.285A; PI 424.353; and PI 424.611A from more than 3400 accessions from the USDA Soybean Germplasm Collection. Bachman et al. (1997) screened 559 soybean accessions from China and found 13 accessions with resistance to BSR. Most of the publicly released BSR resistant cultivars and breeding lines are derived from PI 84946-2, including BSR101 which has the *Rbs₃* allele (Eathington et al., 1995). Under conditions where *P. gregata* infection affects yield, Sebastian et al. (1985) found that in soybean lines derived mostly from PI 84946-2, BSR resistance was associated with a 12 to 16% yield advantage.

Molecular markers close to a gene of interest may be useful for selection in breeding programs, especially for agronomic traits which are difficult to analyze, e.g., disease resistance, insect resistance, and quantitative traits (Lawson et al., 1997; Mohan et al., 1997; Heer et al., 1998). Selection of genotypes resistant to BSR by inoculating plants with isolates of *P. gregata* is laborious and time-consuming. Moreover, assessment of BSR incidence is rendered difficult by seasonal and environmental variation (Nicholson et al., 1973). Soybean breeding efforts to transfer BSR resistance to improved cultivars or soybean lines have been hampered by the low heritability ($h^2 = 0-0.38$) of the trait (Sebastian et al., 1985). Several examples of the application of molecular markers in breeding programs have been presented. Simple sequence repeat (SSR) markers have been used for assessing heterosis in rice breeding (Liu and Wu, 1998). Random amplified polymorphic DNA (RAPD) and sequence characterized amplified region (SCAR) markers were utilized to characterize anthracnose resistance in common bean (Young et al., 1998) and rust resistance in sunflower (*Helianthus annuus* L.; Lawson et al., 1998).

Marker-assisted selection (MAS) could facilitate the development of BSR resistant genotypes. MAS is more efficient than selection based on the phenotype for a trait with low heritability (Van Berloo and Stam, 1998). Gene introgression can readily be followed using molecular markers, which are not influenced by the environmental conditions in which plants are grown. Lewers et al. (1999) identified and mapped molecular markers linked with BSR resistance in the soybean cultivar BSR 101. This study is a follow-up to Lewers et al. (1999) in an attempt to develop breeder-friendly markers. Here we report the development and evaluation of nine new

K.L.E. Klos, M.M. Paz and L. Fredrick Marek, Dep. of Agronomy, Iowa State Univ., Ames, IA 50011; R.C. Shoemaker, USDA-ARS-CICGR and Dep. of Agronomy and Dep. of Zoology/Genetics, Iowa State Univ., Ames, IA 50011; P.B. Cregan, USDA-ARS, Soybean and Alfalfa Research Lab., Beltsville, MD 20705. Research supported by Iowa Soybean Promotion Board. Contribution of the North Central Region USDA-ARS, Project 3236 of the Iowa Agric. and Home Economics Stn. (Journal Paper no. J-18668), Ames, IA 50011-1010. Names are necessary to report factually on the available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable. Received 19 Nov. 1999. *Corresponding author (rcsshoe@iastate.edu).

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Abbreviations: BSR, brown stem rot; MAS, marker-assisted selection; PCR, polymerase chain reaction; PI, plant introduction; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line; SSR, simple sequence repeat.

Table 1. BSR resistant and susceptible germplasm analyzed for the nine PCR-based markers. The allele(s) responsible for BSR resistance is given in parentheses when known.

BSR resistant genotypes†	[Allele(s)]	BSR susceptible genotypes†	BSR susceptible ancestral genotypes†
Acme		A3127	Lincoln
Amsoy		Adams	Mandarin Ottawa
Anoka		Beeson	Ogden
Archer	(<i>Rbs₃</i>)	Blackhawk	Roanoke
BSR 101	(<i>Rbs₃</i>)	Bonus	
BSR 201	(<i>Rbs₃</i>)	Calland	
BSR 301	(<i>Rbs₃</i>)	Capital	
BSR 302	(<i>Rbs₃</i>)	Century	
Elgin 87		Clark	
Grant		Corsoy	
IA 2008	(<i>Rbs₃</i>)	Dorman	
IA 3004		Elgin	
IA 1006		Ford	
L78-4094	(<i>Rbs₁</i>)	Hawkeye	
PI 437.833	(<i>Rbs₂</i>)	Hood	
PI 437.970	(<i>Rbs₃</i>)	IA 2021	
PI 84946-2	(<i>Rbs₁</i> and <i>Rbs₃</i>)	Iroquois	
		Kent	
		Kenwood	
		Parker	
		Pella	
		PI 437.654	
		Shelby	
		Sturdy	
		Wayne	

† The Germplasm Resources Information Network (GRIN), 1999.

DNA markers that can detect BSR resistance in a diverse range of soybean germplasm and discuss their utility in soybean breeding programs.

MATERIALS AND METHODS

Genomic DNA Extraction

Forty-six BSR resistant or susceptible genotypes (Table 1) were identified by querying GRIN data [The Germplasm Resources Information Network (GRIN), 1999] through SoyBase ACEDB version 4.3 (<http://genome.cornell.edu/cgi-bin/WebAce/webace?db=soybase>; verified April 26, 2000). Most BSR resistant genotypes were derived from PI 84946-2 and possess the *Rbs₃* or *Rbs₁* allele. Cultivars and PIs with other sources of resistance were also included (Table 1). Seed for each genotype was obtained from R. Nelson, curator of the USDA Soybean Germplasm Collection, Urbana, IL, or from the R. Shoemaker laboratory, Dept. of Agronomy, Iowa State University, Ames, IA. Seedlings were grown in the greenhouse and DNA was isolated by a method adapted from Saghai-Maroo et al. (1984). The first trifoliolate was harvested, freeze-dried, and ground. The DNA was extracted from 750 mg dried tissue with CTAB buffer followed by chloroform:isoamyl alcohol (24:1) separation and precipitated with 2/3 volume isopropanol, rinsed with 80% (v/v) ethanol:15 mM ammonium acetate solution. After being air-dried, the DNA was resuspended in 1× TE (Tris-EDTA) buffer.

PCR Primer Design

PCR primers were selected from DNA sequences by OLIGO software (National Biolabs, St. Paul, MN). Oligonucleotide primers for K375.sp1 and BSR3.sp1 were designed by means of the DNA sequences of RFLP probes K375 and RGA2, respectively.

The Gm_ISb001 soybean genomic library (Marek and Shoemaker, 1997) was probed with the K375 RFLP probe to identify bacterial artificial chromosome (BAC) clones having

homology to the region of interest. The BACs identified were sequenced from both ends and these sequences were used to develop primers for PCR. PCR amplification products were evaluated for fragment size polymorphism between BSR101 and PI437.654. PCR products not polymorphic in amplification fragment size were screened for restriction site polymorphisms by restriction enzyme digests. Markers polymorphic between BSR101 and PI437.654 were considered for further evaluation of their utility in detecting polymorphism at the *Rbs₃* locus. Satt244, a SSR marker, was developed according to procedures described in Akkaya et al. (1995) and Cregan et al. (1999). Soybean SSRs were developed both from SSR containing sequences available in GenBank and from genomic subclones of Williams soybean DNA. SSR containing subclones were identified by colony hybridization screening using labeled oligonucleotide probes. Positive clones were re-screened and then sequenced to locate the SSR. Primers were developed for more than 600 SSR markers including Satt244. The primers were tested against Williams DNA and 10 additional soybean genotypes. Primers that identified a polymorphism between *G. max* (A81-356022) and *G. soja* (PI 468.916) were mapped in a F2-derived mapping population. Because Satt244 mapped to a region of linkage group J identified by Lewers et al. (1999) to be significantly correlated with BSR resistance in BSR101, it was chosen for further testing to screen for resistance in a wide range of germplasm.

PCR Reaction Conditions

PCR reactions for the BSR3.sp1, K375.sp1, 14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, 35E22.sp1, and 98P22.sp2 markers were carried out in a 20-μL reaction mixture containing 60 ng of genomic DNA, 0.5 μM of each primer, 1× Gibco-BRL PCR buffer, 1.5 mM MgCl₂, 100 μM each of dGTP, dTTP, dATP and dCTP, 0.5 U Taq Polymerase (Gibco-BRL), and 0.5× SCR dye [6% (w/v) sucrose, 100 μM cresol red]. The PCR conditions for BSR3.sp1 and 35E22.sp1 consisted of 94°C for 2 min followed by 35 cycles of 94°C for 1 min (denaturation), 58°C for 45 s (annealing), 72°C for 1 min (extension), and a final extension at 72°C for 5 min. PCR conditions for K375.sp1, 14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, and 98P22.sp2 were as described above with the exception of the annealing temperatures which were as follows: for K375.sp1, 14H13.sp1 and 30L19.sp1 the annealing temperature was 56°C; and for 21E22.sp1, 21E22.sp2, and 98P22.sp1 it was 62°C. Amplification products of 14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, 35E22.sp1, and 98P22.sp2 were digested with *RsaI*, *MspI*, *HhaI*, *Hsp92II*, *HhaI*, and *EcoRI* restriction enzymes, respectively, at 2 U/μL for 1.5 h at 37°C.

SSR analyses were carried out in 20-μL reactions with 60 ng of genomic DNA, 0.15 μM of each primer, 1× Gibco-BRL PCR buffer, 2 mM MgCl₂, 200 μM each of dGTP, dTTP, dATP and dCTP, 0.75 U Taq Polymerase (Gibco-BRL), and 0.5× SCR dye [6% (w/v) sucrose, 100 μM cresol red]. The thermal cycling conditions for the SSR assay were 94°C for 1 min followed by 45 cycles of 94°C for 30 s, 47°C for 30 s, and 68°C for 30 s.

Amplification and digestion products of these markers were separated using a 2% (w/v) agarose gel in 1× TAE (Tris/acetate/EDTA) and visualized by ethidium bromide staining. The samples were electrophoresed for 3 h at 90 V.

Molecular Marker Evaluation

PCR and enzyme digest products were compared to determine the efficacy of distinguishing BSR resistance in different

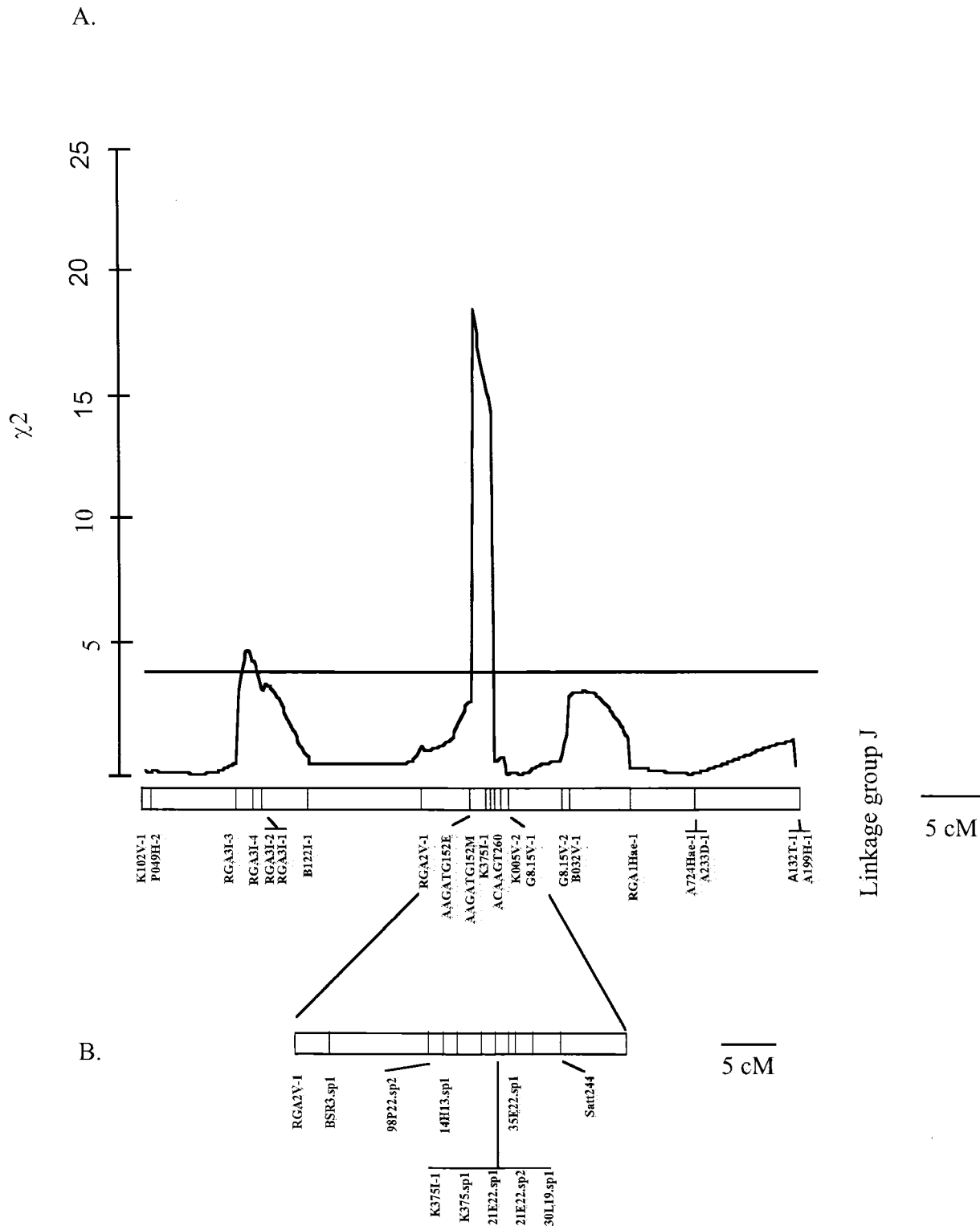


Fig. 1. Soybean Linkage Group J from the BSR101 by PI437.654 recombinant inbred line population showing: **A.** Marker association with brown stem rot resistance as measured by foliar disease severity, and **B.** Map locations of new markers in relation to RGA2V-1 and K375L-1. Associations are illustrated by a curve from QTL Cartographer. The horizontal bar indicates significance at $P = 0.05$. Adapted from Lewers et al. (1999).

cultivars and PIs. Restriction enzyme recognition site polymorphisms and polymorphic amplification products were observed between the parents of several mapping populations including the parents of the population segregating for brown stem rot resistance, BSR 101 and PI 437.654. The gene diver-

sity of a locus, defined by Weir (1990) as the amount of polymorphism in homozygous progeny of a self-fertilizing species, has been used as an estimator of the polymorphism information content (PIC) value of a molecular marker (Anderson et al., 1992). The PIC value of a PCR-based marker was calculated as

Table 2. Primer sequences for DNA markers associated with BSR resistance

Marker	Primer 1	Primer
BSR3.sp1	5'-CGATTGGTTTGGTTCTGGC-3'	5'-TTTCATATAGCATGGATCAAC-3'
K375.sp1	5'-ACCATTAGGACTGAGTTTG-3'	5'-GCTTGAATAGCGATCCTTC-3'
14H13.sp1	5'-GTCACACACAAATTCCTAG-3'	5'-TGGGTGTAGTCCGGGTG-3'
21E22.sp1	5'-GCTTTTGCTCCGTTCAAGTCC-3'	5'-GGCCACTCTCACCGATCT-3'
21E22.sp2	5'-GCTTTTGCTCCGTTCAAGTCC-3'	5'-GGCCACTCTCACCGATCT-3'
30L19.sp1	5'-GAAGCTAATACGCCATAAAC-3'	5'-CTTCACAGTCCCTTTTCAC-3'
35E22.sp1	5'-ACACTGTTTGGGACCGAATCA-3'	5'-ATAGAAAGAGCCCATCCGATAA-3'
98P22.sp2	5'-TGGAGATCATTTGGCTGT-3'	5'-ACTGAAAGGTCGGGTAAA-3'
Satt244	5'-GCGCCCCATATGTTTAAATTATATGGAG-3'	5'-GCGATGGGGATATTTCTTTATTATCAG-3'

adapted by Weir (1990, p. 125) from Nei (1987, p. 106–107):

$$1 - \sum_{j=1}^n P_{ij}^2$$

where P_{ij} is the frequency of the j th PCR pattern for Genotype i .

In addition, PCR analyses using all nine markers were done on a recombinant inbred line (RIL) population derived from a cross between BSR 101 and PI 437.654 (Baltazar and Mansur, 1992) which are resistant and susceptible to BSR, respectively. RILs were screened for BSR resistance by Lewers et al. (1999). For mapping purposes, the banding patterns in the parental genotypes and in the RILs were scored as A or B in 320 RILs. The markers were added to the map reported by Lewers et al. (1999) by Mapmaker 2.0 with the default parameters LOD 3.0 and maximum recombination of 30%. The 'TRY' and the 'RIPPLE' commands were used to confirm the map (minimum LOD score of 2.0, window size of 3).

RESULTS

Marker Identification

The method of location-specific molecular marker development, utilizing DNA sequences from RFLP probes and BACs, was successful at generating markers which mapped to the region of interest on soybean linkage group J (Fig. 1B). Twenty-nine PCR primer sets developed from BAC end sequences were discarded from further evaluation in this study due to lack of polymorphism between BSR101 and PI437.654. The markers BSR3.sp1, and K375.sp1 (Table 2), developed from RFLP probe sequences were polymorphic in PCR amplification size between BSR101 and PI437.654. Two PCR primer sets developed from BAC sequences were observed to amplify fragments polymorphic in size between BSR101 and PI437.654 (data not shown), but these polymorphisms were not reproducible under stringent PCR conditions and so were discarded from further evaluation. Polymorphism between BSR101 and PI437.654 was observed in six markers (14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, 35E22.sp1, and 98P22.sp2) developed from BAC end sequences after restriction enzyme digest of the PCR product (Table 2). This study demonstrates the utility of BAC library sequences in conjunction with an experimental population segregating for the gene of interest as a source of new markers that are polymorphic among a large group of genotypes.

Segregation Analysis

RILs derived from a cross between BSR 101 and PI 437.654 were analyzed to confirm the usefulness of

markers to monitor BSR resistance during inbreeding, i.e., to confirm linkage with *Rbs3*. A total of 320 RILs were assayed with BSR3.sp1, K375.sp1, 14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, 35E22.sp1, 98P22.sp2, and Satt244. The marker scores were used to map the nine new markers against one another and to place them in relation to the molecular genetic map reported by Lewers et al. (1999) with the same set of RILs. Lewers et al. (1999) mapped markers associated with one major and one minor QTL in linkage group J (Fig. 1A). A major gene (*Rbs3*) and a second gene with a minor effect control BSR resistance in BSR101 (Eathington et al., 1995). We believe that markers identified in this study are at the major QTL (*Rbs3*) that was mapped by Lewers et al. (1999) between RGA2V-1 and G8.15V-1 of linkage group J (Fig. 1). BSR3.sp1 was mapped near marker RGA2V-1. The K375.sp1, 14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, 35E22.sp1, and 98P22.sp2 markers mapped within the cluster of markers AAGATG152E, AAGATG152M, K375I-1, and ACAAGT260. Satt244 was mapped near the RFLP markers K005V-2 and G815V-1. All of these markers are in the region of linkage group J identified to have the maximum correlation with BSR resistance controlled by *Rbs3*, in BSR 101 (Fig. 1; Lewers et al., 1999).

The BSR3.sp1, K375.sp1, 14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, 35E22.sp1, 98P22.sp2, and Satt244 markers were successful at differentiating among resistant and susceptible RILs. Three hundred twenty RILs were inoculated with *Phialophora gregata* in a glasshouse by Lewers et al. (1999) and rated for foliar disease severity from 0 (healthy) to 10 (all leaflets dead or missing). We compared their foliar severity results with our marker evaluation of the RIL population. Figure 2 shows the number of RILs within each BSR disease rating that were scored for the 'A' allele (derived from the resistant parent) or the 'B' allele. This figure indicates the number of RILs which would have been incorrectly classified as resistant by the marker allele score as the selection criteria. For example BSR3.sp1 identified 148 RILs as potentially resistant on the basis of the 'A' allele, but 41 of these have disease severity ratings of 5 or greater (susceptible to highly susceptible). 30L19.sp1 identified 132 potentially resistant RILs, and 34 of these were rated 5 or greater in the greenhouse disease severity screen. A set of 44 RILs was identified as highly resistant and a set of 49 RILs as highly susceptible to BSR based on foliar symptoms in relation to the parental genotypes (Lewers et al., 1999). These markers were able to identify highly resis-

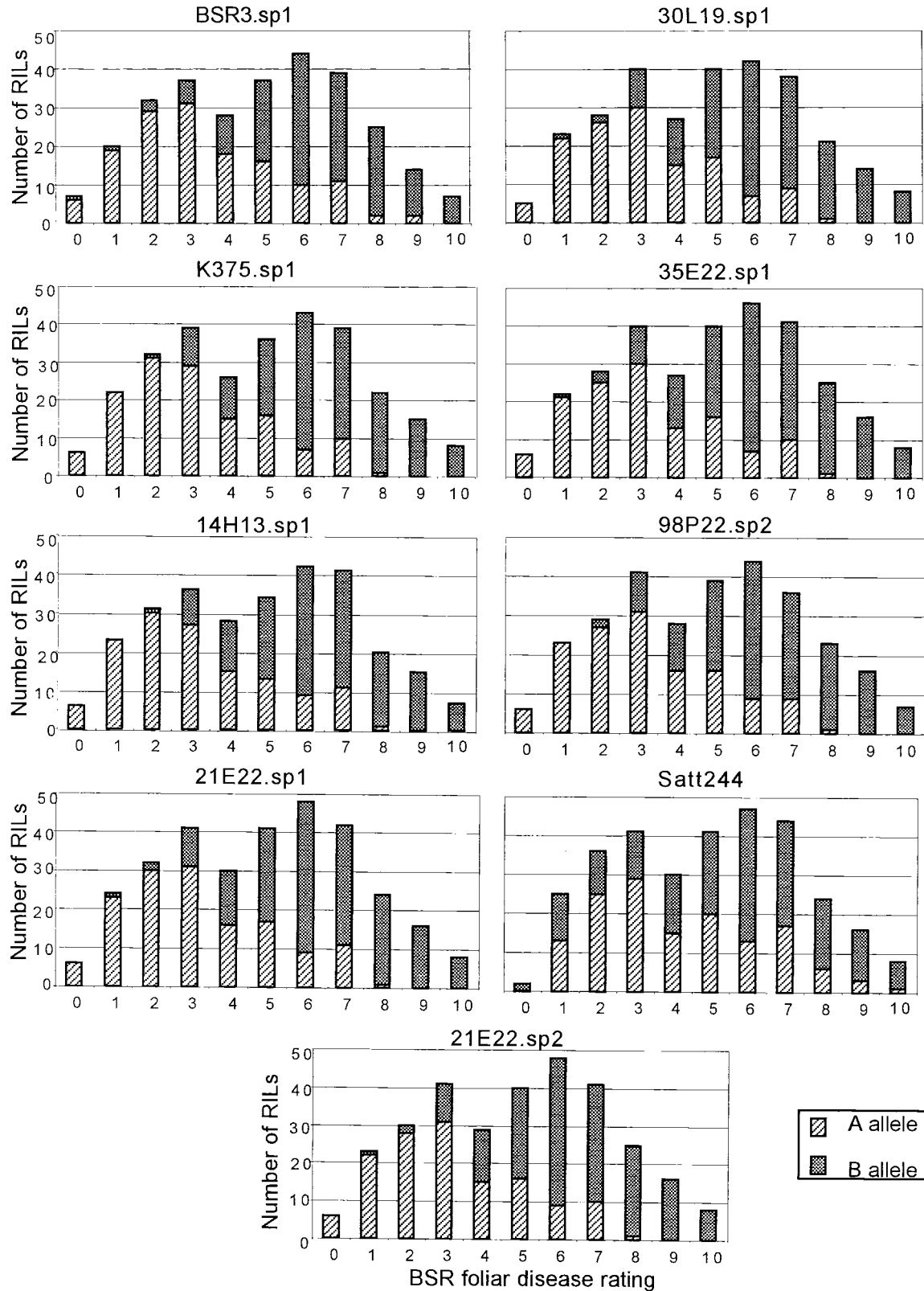


Fig. 2. BSR foliar disease severity ratings (0 = healthy to 10 = most severe) (x axis) and the number of RILs possessing the 'A' allele or the 'B' allele (y axis) for BSR markers BSR3.sp1, K375.sp1, 14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, 35E22.sp1, 98P22.sp2, and Satt244. The 'A' allele corresponds to that derived from the resistant parent. The 'B' allele corresponds to that derived from the sensitive parent.

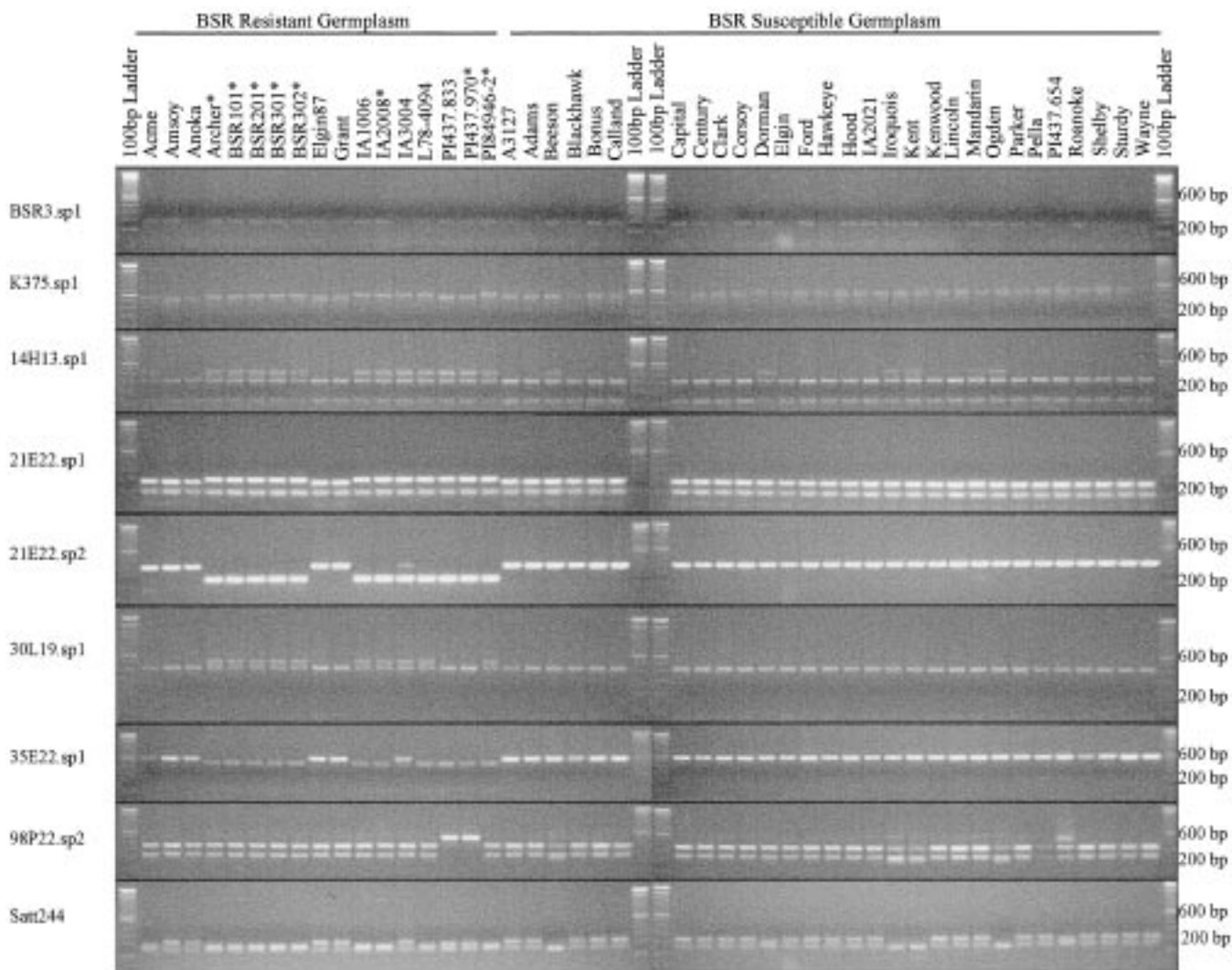
Table 3. Polymorphism information content (PIC) values and frequency of BSR101 parental allele ('A') in 44 recombinant inbred lines scored as highly resistant to brown stem rot on the basis of foliar symptoms; the frequency of PI437.654 parental allele ('B') in 49 lines scored as highly susceptible, for nine DNA markers on the basis of 46 genotypes.

Marker	PIC	Frequency of 'A' in resistant RILs	Frequency of 'B' in susceptible RILs
BSR3.sp1	0.38	0.91	0.86
K375.sp1	0.52	0.98	0.96
14H13.sp1	0.49	0.95	0.96
21E22.sp1	0.38	0.98	0.98
21E22.sp2	0.39	0.98	0.98
30L19.sp1	0.34	0.98	0.96
35E22.sp1	0.36	0.95	0.98
98P22.sp2	0.27	0.98	0.93
Satt244	0.57	0.93	0.98

tant genotypes with an accuracy of 90% or greater, and susceptible genotypes with a greater than 85% accuracy (Table 3). These markers will be particularly useful for monitoring soybean populations segregating for *Rbs3*.

Evaluation in Soybean Germplasm

The DNA markers, BSR3.sp1, K375.sp1, 14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, 35E22.sp1, 98P22.sp2, and Satt244, which were developed on the basis of polymorphism between BSR101 and PI437.654, were evaluated in a set of cultivars, PIs, and ancestral genotypes identified as resistant or susceptible to brown stem rot on the basis of GRIN data (Fig. 3). The markers differed in the degree of polymorphism observed among the set of genotypes evaluated. The PIC values (Table 3) signify the possible usefulness of the markers as a means of detecting a polymorphism between two soybean cultivars. The largest PIC value was observed for Satt244 and the smallest for 98P22.sp2. A larger PIC value indicates a greater likelihood that polymorphism will be observed between any two genotypes. In a soybean breeding program to transfer BSR resistance due to the *Rbs3* gene, a susceptible cultivar could be used as one parent and a resistant cultivar with a dissimilar PCR banding pattern could be used as the other parent. The



* Presumed to contain the *Rbs3* allele for resistance to BSR.

Fig. 3. Amplification banding patterns of BSR3.sp1, K375.sp1, 14H13.sp1, 21E22.sp1, 21E22.sp2, 30L19.sp1, 35E22.sp1, 98P22.sp2, and Satt244 markers in 46 soybean cultivars and PIs which are resistant or susceptible to BSR.

marker 35E22.sp1 had the second lowest PIC value, yet it is apparent in a comparison of the banding patterns of resistant and susceptible genotypes that this marker may, along with 21E22.sp1, 21E22.sp2, and 30L19.sp1, be one of the most useful as predictor of resistance in a germplasm screening program (Fig. 3). None of the markers differentiated among the different genes for BSR resistance. Many of the BSR resistant soybean lines included in this study have the *Rbs₃* allele (Table 1). Soybean lines L78-4049 and PI 437.833 have BSR resistance alleles *Rbs₁* and *Rbs₂*, respectively; and PI 84946-2 has both *Rbs₁* and *Rbs₃* (Eathington et al., 1995; Hanson et al., 1988; Willmot and Nickell, 1989; Sebastian and Nickell, 1985). The source of BSR resistance in the remainder of the lines is unknown, but may be due to the presence of one or more alleles for BSR resistance, possibly including *Rbs₃*. No marker or combination of markers from this set could be identified which would differentiate among resistant lines with different alleles (Fig. 3). Therefore, the use of these markers in a breeding program for BSR resistance requires a parent whose resistance is known to be due to the *Rbs3* gene, or a test of linkage between resistance and the marker in the segregating progeny. For example, a marker screening program in the progeny of a cross between L78-4094 and any of the susceptible genotypes in Fig. 3, determined on the basis of the polymorphic 35E22.sp1 marker, would not select BSR resistant lines because L78-4094 is resistant due to the *Rbs1* allele (Table 1).

The greenhouse or field screening procedure for evaluating BSR resistance involves inoculating plants with the causal pathogen and obtaining foliar and stem ratings for disease severity. This method is lengthy, often involves destructive sampling, and disease symptoms are affected by environmental conditions. Our objective was to develop breeder-friendly markers for efficient identification of BSR resistant plants in any soybean population possessing one of the major BSR resistance genes. The markers developed in this study will most likely be useful for screening BSR resistance and allow soybean breeders to rapidly transfer resistance derived from *Rbs₃* to improved cultivars or new and improved soybean lines. The markers described here are easy-to-use, inexpensive, and highly informative. These markers may also be used to more precisely identify the location of the resistance gene for the purpose of map-based cloning.

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Simple Sequence Repeat Diversity among Soybean Plant Introductions and Elite Genotypes

James M. Narvel, Walter R. Fehr,* Wen-Chy Chu, David Grant, and Randy C. Shoemaker

ABSTRACT

The use of molecular markers to facilitate the introgression of plant introduction (PI) germplasm into elite soybean [*Glycine max* (L.) Merr.] cultivars will depend on the amount of polymorphism that exists between elite genotypes and PIs. The objective of this study was to assess the simple sequence repeat (SSR) diversity of 39 elite soybean genotypes (Elites) and 40 PIs that were selected for high yield potential. A total of 397 alleles were detected among the 79 genotypes at 74 SSR marker loci. The number of alleles detected among the PIs was 30% greater than that detected among the Elites. There were 138 alleles specific to the PIs that occurred across 60 SSR loci and 32 alleles specific to the Elites that occurred across 27 SSR loci. Average marker diversity among the PIs was 0.56 and ranged from 0.0 to 0.84. Average marker diversity among the Elites was 0.50 and ranged from 0.0 to 0.79. Genetic similarity estimates based on simple matching coefficients revealed more genetic diversity among the PIs than among the Elites. The greatest genetic diversity was between the PIs and Elites. The ability of SSRs to distinguish among elite soybean genotypes and PIs with agronomic merit may assist with the transfer of favorable alleles from PIs into elite soybean cultivars.

THE LIMITED GENETIC BASE of North American soybean cultivars is due to the contribution of fewer than 20 plant introductions (PIs) to the primary gene pool and to the repeated use of related parents in breeding programs (Gizlice et al., 1994). Expanding the genetic base of soybean may introduce unique favorable alleles for polygenic traits. It is not possible at present to evaluate directly alleles for polygenic traits in soybean; therefore, incorporation of PIs with agronomic merit into breeding programs has been used as an alternative strategy (Thorne and Fehr, 1970; Vello et al., 1984; Thompson and Nelson, 1998). It is not known if selection of PIs for agronomic potential affects their diversity relative to elite germplasm. Because most PIs have no known pedigree, the genetic diversity among PIs or between PIs and elite genotypes (Elites) cannot be estimated by a coefficient of parentage analysis.

DNA marker analysis is an alternative method of

estimating the diversity of PIs that are candidates as parents in a breeding program. The hypothesis is that the more genetically diverse the PIs are from the elite parents, the more likely they are to possess unique alleles for traits of interest. Several studies have measured the diversity of PIs and Elites with restriction fragment length polymorphism (RFLP) markers. Greater diversity has been detected in PIs than in Elites, but the level of polymorphism has been low (Keim et al., 1989; Keim et al., 1992). Amplified fragment length polymorphic (AFLP) and random amplified polymorphic DNA (RAPD) markers have been shown to be more polymorphic in soybean than RFLPs (Powell et al., 1996). Maughan et al. (1996) used 15 primer pairs for AFLP analysis of a broad sample of 23 soybean accessions including *G. max* and wild (*Glycine soja* Sieb. and Zucc.) genotypes. Of the 759 AFLP fragments detected in their study, 36% were polymorphic across all genotypes. Within the group of *G. soja* genotypes, 31% were polymorphic. Only 17% were polymorphic within the *G. max* group that included four PIs and 12 elite genotypes. Thompson et al. (1998) used 125 primers for RAPD analysis of 18 soybean ancestral lines and 17 PIs of Maturity Group I to III that were selected for their seed yield. They reported that 34% of the amplified fragments detected were polymorphic across the 35 genotypes and indicated that this marker system may be useful for introgressing favorable alleles from PIs into elite breeding populations.

Simple sequence repeat (SSR) DNA markers have been shown to be highly polymorphic in soybean (Akkaya et al., 1992; Diwan and Cregan, 1997). SSRs are composed of a 1- to 6-base pair (bp) DNA sequence that is repeated a variable number of times. SSRs are amplified by PCR with primers that are complementary to the conserved sequences that flank an SSR locus. Polymorphic fragments (alleles) resulting from variations in SSR repeat length are separated electrophoretically to display genetic profiles of individuals. SSR alleles typically show monogenic-codominant inheritance that enables classification of homozygotes and heterozygotes in a segregating population.

Akkaya et al. (1992) used several types of SSRs to

J.M. Narvel and W.R. Fehr, Dep. of Agronomy; Wen-Chy Chu, DNA Sequencing and Synthesis Facility; and David Grant and R.C. Shoemaker, USDA-ARS-CICG, Dep. of Agronomy, Iowa State University, Ames, IA 50011. Journal Paper No. 18637 of the Iowa Agric. and Home Econ. Exp. Stn., Ames, IA 50011. Project No. 3107, and supported by the Hatch Act, the State of Iowa, and the Iowa Soybean Promotion Board. Received 13 Oct. 1999. *Corresponding author (wfehr@iastate.edu).

Abbreviations: AFLP, amplified fragment length polymorphism; bp, base pair; cM, centimorgan; LG, linkage group; MG, maturity group; RAPD, random amplified polymorphic DNA; QTL, quantitative trait loci; SMC, simple matching coefficient; SSR, simple sequence repeat.